

Remarks

Claims 54-72 are pending in the subject application. By this Amendment, Applicants have added claims 73-85, amended claim 65 and canceled claims 54-64 and 69-72. Support for the newly presented claims can be found in the originally presented claims and at pages 2-5 and 12-18, for example. Entry and consideration of the amendments and new claims presented herein is respectfully requested. Accordingly, claims 65-68 and 73-85 are currently before the Examiner (with claims 66-67 and 76-77 standing withdrawn from consideration as being directed to a non-elected invention). Claims 65, 68, 73-75 and 78-85 read on the elected species. Favorable consideration of the pending claims is respectfully requested.

Submitted herewith is a supplemental Information Disclosure Statement (IDS), accompanied by the form PTO/SB/08, and copies of the references listed herein. Applicants request that the references in the IDS be made of record in the subject application.

Claims 55 and 56 were objected to for various informalities. Applicants thank the Examiner for his careful review of the claims and the suggested corrections made in the Office Action. The corresponding claims that have been newly presented in this response have been amended to attend to the formalities noted in the Office Action. Accordingly, reconsideration and withdrawal of the objection is respectfully requested.

Claim 56 was rejected under 35 U.S.C. § 112, second paragraph, as indefinite as including diseases or conditions that are not conditions typically associated with tumors or precancerous growths. Applicants have amended newly presented claim 75 to remove these conditions and reconsideration and withdrawal of the rejection under 35 U.S.C. § 112, second paragraph, is respectfully requested.

Claims 54-56, 59, 60, 65, and 68 are rejected under 35 U.S.C. § 112, first paragraph, as nonenabled by the subject specification. The Office Action articulates two primary reasons for instituting this rejection and, to the extent that this rejection may be applied to the newly presented claims, Applicants provide the following response. The first aspect of the rejection relates to the argument that “it is considered highly unpredictable as to what the effects of PRF1 expression inhibition would have been *in vivo* in tumor or precancerous cells”. The second aspect of the rejection relates to an argument that the as-filed specification “does not overcome art recognized

obstacles to *in vivo* RNAi, particularly in terms of specific targeting and delivery of the dsRNA to a whole organism. As a result, one of skill in the art would have to perform undue experimentation in order to practice the claimed invention.” Applicants respectfully traverse this rejection.

At the outset, Applicants note that the first aspect of the rejection does not appear to have been applied against claims 65 and 68. Confirmation of this assessment is respectfully requested.

In support of its position that it is unpredictable what effects the inhibition of PRF1 expression inhibition would have been *in vivo* in tumor or precancerous cells (Office Action at pages 5-8), the Office Action argues:

The specification as filed teaches that PRF1 seems to be regulated by both the HIF1 alpha and AKT branches of the PI3K network and occurs downstream of mammalian target of rapamycin (mTOR). The specification indicates that a decrease in the expression or activity of PRF1 is suitable to put a cellular system into a condition corresponding to hypoxic conditions, which may lead to apoptosis, so inhibition of PRF1 can stimulate apoptosis for therapeutic purposes in tumor treatment. See pages 12 and 18 (paragraphs 56 and 70 of the published application). However, Fig. 1 of Katso discloses at least two other pathways by which PI3K can inhibit apoptosis (i.e. through stimulation of IKK or inhibition of BAD), and the relationship of these pathways to PRF1 is not known.

The Office Action also presents similar argument at pages 7-8, where it is stated: “... it is considered unpredictable as to what would be the effects of PRF1 expression inhibition in cells generally in view of the complexity of the PI3K signaling network.” Additionally, the Office Action presents a series of references asserted to support a finding that:

In view of the facts that, at the time of the invention, the control of the cell cycle by PI3K-mediated events was considered to be extremely complicated, the role of PRF1 in this process was incompletely understood, the biochemical function of PRF1 was unknown, and PRF1 was observed to elicit opposite effects on cell survival in different contexts, it is considered to be highly unpredictable as to what the effects of PRF1 expression inhibition would have been *in vivo* in tumor or precancerous cells generally. This unpredictability is borne out in the post filing art (DeYoung, Lapointe, above) which suggests that PRF1 is a tumor suppressor in breast tumor cells, and is underexpressed in primary prostate carcinoma cells. Accordingly, one of skill in the art at the time of the invention could not have known generally in which primary tumor or precancerous cells *in vivo* the invention would function as claimed. Because the specification and prior art do not provide information as to how to predict which tumor or precancerous cells will be inhibited

by PRF1 siRNA, one of skill in the art would have had to perform undue experimentation in order to practice the invention as claimed.

In this regard, Applicants respectfully submit that those skilled in the art, at the time the invention was made, would have been aware of the complexity of the PI3K network, especially in view of Katso, Figure 1. Additionally, those skilled in the art would also have been aware that PI3K could possibly inhibit apoptosis via stimulation of IKK or inhibition of BAD. However, it is respectfully submitted that one skilled in the art would have been able to both assess and counteract any possible role of PI3K mediated inhibition of apoptosis through stimulation of IKK or inhibition of BAD.

Additionally, Applicants also submit that the as-filed specification provides evidence that the inhibition of PRF1 was able to reduce tumor volume *in vivo* and metastatic activity of cancer cells *in vitro* (see, for example, Examples 9-10 and Figures 6C-D and Figures 11A-B). Additionally, the as-filed specification provides evidence that PRF1 is expressed in prostate tumor tissue (Example 7 and Figure 8B). Thus, it is respectfully submitted that the as-filed specification provides evidence that inhibition of PRF1 can result in inhibition of the growth of a tumor or precancerous growth as well as inhibit the metastatic or migrational activity of cancer cells. Additionally, these results would appear to indicate that the other pathways by which PI3K can, possibly, inhibit apoptosis (*i.e.*, through stimulation of IKK or inhibition of BAD) do not appear to affect the ability of PRF1 to mediate this effect. Accordingly, it is respectfully submitted that one skilled in the art would have been enabled to practice the claimed invention in view of the teachings of the as-filed specification.

The Office Action also argues that “[i]t is clear that not all aspects of the PI3K network are functional in all cells, so it follows that tumors that are caused by defects in separate PI3K pathways that are not functional in the same cell would not be treatable by an agent that affects only one of the pathways.” Applicants respectfully submit that the presently pending claims are directed to inhibiting the growth of a tumor or a precancerous growth associated with dysregulation of phosphoinositide 3-kinase (PI-3) signaling and hyperactivation of the HIF-1 $\alpha$  signaling pathway and/or hyperactivation of the AKT signaling pathway. As noted in the last Office Action and this response, the as-filed specification and examples indicate that PRF1 appears to be regulated by both HIF-1 $\alpha$  and AKT. Additionally, the as-filed specification indicates that “as HIF1 alpha is up-

regulated under hypoxic conditions and Akt [is] up-regulated under stress conditions to trigger a survival response which is counter-acting the apoptosis reaction of a cell, a compound addressing PRF1 as described herein and which may also be used as medicament or interaction partner of PRF1, both at the nucleic acid level and/or the protein level, can transfer the cell into a condition, whereupon other compounds are particularly effective” (see page 18, first full paragraph). Thus, it is respectfully submitted that the as-filed specification fully enables the claimed invention and that one skilled in the art would have been able to inhibit the growth of a tumor or a precancerous growth associated with dysregulation of phosphoinositide 3-kinase (PI-3) signaling and hyperactivation of the HIF1- $\alpha$  signaling pathway and/or hyperactivation of the AKT signaling pathway.

The Office Action also argues that the effects of manipulating PRF1 expression were unpredictable and would have to be determined in a case by case basis for different tumors (citing to various references, such as DeYoung *et al.*, LaPointe *et al.*, Faris *et al.*, Riggins *et al.* and Monahan *et al.* in support of this position). Applicants submit that the Patent and Trademark Office Board of Patent Appeals and Interferences has stated: “The test [for enablement] is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed to enable the determination of how to practice a desired embodiment of the invention claimed”. *Ex parte Jackson*, 217 U.S.P.Q. 804, 807 (1982); *see also Ex parte Erlich* 3 U.S.P.Q.2d 1011 (B.P.A.I. 1982) (observing that although a method might be “tedious and laborious”; such experimentation is nevertheless “routine” defining “routine” experiments as those which use known methods in combination with the variables taught in the patent to achieve the expected, specific, patented result).

In this regard, Applicants have taught that inhibition of PRF1 results in inhibition of tumor growth as well as inhibition of metastatic or migrational activity by tumor cells. Applicants have also taught both *in vitro* and *in vivo* systems in which one can assess the effects of PRF1 inhibition on tumor growth and/or metastatic or migrational activity (Examples 9-10). Furthermore, the ability of PRF1 to inhibit tumor growth and/or metastatic or migrational activity could have been assessed on any number of cell lines that would have been available to those skilled in the art at the time the invention was made or on actual tumor cells extracted from patients in order to assess the sensitivity

of the extracted cells to PRF1 inhibition. Such experimentation may have been "tedious and laborious," such experimentation, nevertheless, would have been "routine" in view of the teachings of the specification and various methods known in the art at the time the invention was made. Thus, it is respectfully submitted that the as-filed specification enables the claimed invention and reconsideration and withdrawal of this aspect of the invention is respectfully requested.

The second aspect of the enablement rejection in this matter is directed to the delivery of siRNA molecules *in vivo*. The Office Action argues that:

Those of skill in the art at the time of the invention, and after the invention, recognized significant obstacles related to the predictability of inhibiting expression of a target gene *in vivo* by RNA interference (RNAi), particularly in regards to the *in vivo* targeting and delivery of specific nucleic acids that mediate RNAi to the appropriate cell/organ, at a bio-effective concentration and for a period of time such that said molecule is effective in inhibiting expression of a target gene. Indeed, nucleic acid based therapies at the time of filing were highly unpredictable and while it is recognized that introduction of dsRNA targeted to a specific gene may result in expression inhibition, the successful delivery of dsRNA to a target cell *in vivo*, such that the requisite biological effect was provided to the target cells/tissues/organs, must be determined empirically.

In support of this position, the Office Action cites to a number of references asserted to support this position.

For example, the Office Action states:

Opalinska et al. (Nature Reviews Drug Discovery, 2002, Vol. 1, pp. 503-514) stated, "[I]t is widely appreciated that the ability of nucleic-acid molecules to modify gene expression *in vivo* is quite variable, and therefore wanting in terms of reliability. Several issues have been implicated as a root cause of this problem, including molecule delivery to targeted cells and specific compartments within cells and identification of sequence that is accessible to hybridization in the genomic DNA or RNA", and in column 2 of the same page, "[a]nother problem in this field is the limited ability to deliver nucleic acids into cells and have them reach their target. Without this ability, it is clear that even an appropriately targeted sequence is not likely to be efficient. As a general rule, oligonucleotides are taken up primarily through a combination of adsorptive and fluid-phase endocytosis. After internalization, confocal and electron microscopy studies have indicated that the bulk of the oligonucleotides enter the endosome-lysosome compartment, in which most of the material becomes either trapped or degraded."

However, the same passage cited in the Office Action (page 511, column 2) continues: “Nevertheless, oligonucleotides can escape from the vesicles intact, enter the cytoplasm and then diffuse into the nucleus, where they presumably acquire their mRNA, or in the case of decoys, protein target<sup>131, 132-134</sup>. Delivery technologies continue to improve, so it is likely that present methods, and/or other evolving technologies, will be used to successfully deliver optimized nucleic acids to their cellular targets<sup>135, 136</sup>. Thus, it is clear that this reference is of the opinion that delivery technologies available at the time this application was filed could have been used to successfully deliver nucleic acids, such as siRNA molecules, to their cellular targets.”

The Office Action argues that Caplen (2003) teaches that “[m]any of the problems associated with developing RNAi as an effective therapeutic are the same as encountered with previous gene therapy approaches. The key issues of delivering nucleic acids to the required tissue and cell type, while ensuring an appropriate level of efficacy with minimum toxicity induced by the vector system ...”. (page 581). However, this same reference also indicates: that siRNA molecules can be successfully delivered to target organs, such as liver, spleen, pancreas, lung and kidney (albeit via high pressure tail vein injection in mice); that siRNA were successfully delivered *in vivo* via adenoviral vectors and or AAV-vectors; and that embryonic stem cells and hematopoietic stem cells had been successfully used to mediate RNAi *in vivo* (see page 580, column 2, RNAi *in vivo*).

Coburn *et al.* (2003) is argued as teaching “... that the major impediment to using RNA interference as a therapeutic is that suppression of gene expression is transient and the delivery methods used for RNAi are not effective for therapeutic purposes (see for example p. 754, first column, last paragraph).” However, that same paragraph continues:

An enormous step forward in addressing these complications is the finding that natural or designed siRNAs and miRNAs can be expressed *in vivo*.<sup>30-33</sup> Short hairpin and pre-miRNA-based precursor RNAs can be transcribed from either RNA polymerase III (H1 or U6)<sup>30,32</sup> or RNA polymerase II promoters<sup>33</sup> *in vivo*, and processed by Dicer to release functional siRNAs. Importantly, siRNAs expressed from DNA templates can silence gene expression as effectively as exogenously introduced synthetic siRNAs.<sup>30,31,33</sup> As an extension of these studies, many groups have begun constructing the first generation of retroviral-<sup>34</sup> adenoviral-<sup>35</sup> and lentiviral-based<sup>36</sup> gene therapy vectors that are capable of expressing siRNAs in a stable manner in virtually any cell and tissue type. Already progress has been made, with the demonstration that murine retroviral vectors expressing siRNAs directed

against a mutant allele of the human K-Ras protooncogene have the ability to reverse tumorigenicity in mice.<sup>34</sup>

Applicants note that many of the cited references were published around the time the instant application was first filed and that the passage cited actually would appear to support a finding that one skilled in the art, at the time the instant application was filed, would have been able to practice the claimed invention without undue experimentation.

The Office Action also argues that Check (Nature, 2003, Vol., 425, pp. 10-12) reported:

... scientists must figure out how to make RNAi therapies work. They are facing some formidable technical barriers, chief among which is the problem of getting siRNAs into the right cells. This is not a trivial issue, because RNA is rapidly broken down in the bloodstream and our cells don't readily absorb it through their membranes. And even when RNA gets into its target cell, scavenger proteins quickly chew it up. (see page 11, middle column, second full paragraph). Check describes that delivery methods are of concern to many researchers. In column 2 of page 11: ... "The major hurdle right now is delivery, delivery, delivery" says Sharp and in column 3 of the same page, Khvorova believes that the medical benefits of RNAi will be huge if the delivery issues can be resolved. "But we've looked at a lot of the delivery methods that have been used for antisense, and so far I haven't been impressed," she says.

With respect to this line of argument, Applicants submit that the passage referred to within the cited reference (column 3, lines 8-10) also states that the delivery systems utilized in antisense clinical trials were not very effective. While these delivery systems may not have yielded optimal results in clinical trials, from the perspective of the author and the scientists quoted within the article, the delivery systems were capable of delivering nucleic acids to target cells, albeit not in a particularly effective manner. As the Patent Office is aware, clinical efficacy is not required to enable the claims. *See CFMT, Inc. v. Yieldup Int'l Corp.*, 349 F.3d 1333, 1338, 68 USPQ2d 1940, 1944 (Fed. Cir. 2003) ("Enablement does not require an inventor to meet lofty standards for success in the commercial marketplace."). Thus, it is respectfully submitted that one skilled in the art would have been able to practice the claimed invention without undue experimentation.

The Office Action also argues:

Zhang et al (Current Pharmaceutical Biotechnology 2004, Vol. 5, pp.1-7) reviewed the state of the art with regard to RNAi, and stated "[u]se of siRNA in

mammalian cells could be just as far-reaching, with the applications extending to functional genomics and therapeutics. But various technical issues must be addressed, especially for large-scale applications. For instance, dsRNA can be delivered to *C. elegans* by feeding or soaking, but effective delivery of siRNAs to mammalian cells will not be so simple.”

In particular regards to Applicant’s ex vivo example, often formulations and techniques for delivery in vitro (cell culture) are not applicable in vivo (whole organism). For example, Agrawal et al (Agrawal et al. (Mol. Med. Today 6:72-81, 2000) stated “[t]he cellular uptake of negatively charged oligonucleotides is one of the important factors in determining the efficacy of antisense oligonucleotides.... in vitro, cellular uptake of antisense oligonucleotides depends on many factors, including cell type, kinetics of uptake, tissue culture conditions, and chemical nature, length and sequence of the oligonucleotide. Anyone of these factors can influence the biological activity of an antisense oligonucleotide.” Agrawal discussed these factors in relation to antisense, but they would also apply to dsRNA.

Regarding these references, Applicants recognize that delivery of siRNAs to mammalian cells is not as simple as delivering siRNAs to *C. elegans* via feeding or soaking and that any number of factors can determine the efficacy of a therapeutic protocol. However, Applicants respectfully submit that those skilled in the art, at the time the invention was made, had a number of tools available that would have allowed for delivery of siRNA molecules to target cells or tissues, and clinical efficacy is not required to enable the claims. *See CFMT, Inc. v. Yieldup Int’l Corp.*, 349 F.3d 1333, 1338, 68 USPQ2d 1940, 1944 (Fed. Cir. 2003).

For example, naked siRNA molecules (unmodified siRNA molecules) or siRNA molecules modified to be resistant to nucleases could have been administered to a subject in order to contact tumor cells, precancerous growths and/or migrating/metastasizing tumor cells. Applicants note that the as-filed specification indicates (at page 31) that siRNA molecules can be designed according to the teachings of published International Patent Applications WO 00/44895 and WO 01/75164 each of which discuss methods of making siRNA and siRNA molecules that are resistant to nucleases (see pages 6 and 14, respectively; for the Examiner’s convenience, an English language equivalent of the ‘895 document is AU 200032713 B2 and is provided in the Information Disclosure Statement). These nucleotides could also have been integrated into retroviral vectors for expression within target tissues (see, for example, Devroe *et al.*, *BMC Biotech.*, 2002, 2:15-19). As discussed therein,



retroviral vectors are efficient systems for delivery of siRNA into target cells and these vectors could have been directly administered to cells via systemic administration of loaded onto targeted non-viral gene delivery systems discussed below.

Alternatively, naked or modified siRNA molecules could have been loaded onto targeted non-viral gene delivery systems such as those discussed in Ogris *et al.* (*DDT*, 2002, 7(8):479-485). The reference discusses various DNA condensing systems suitable for delivering DNA to target cells; however, one skilled in the art would have recognized the applicability of such delivery systems to dsRNA molecules, such as siRNA.

As discussed in Ogris *et al.*, a number of non-viral nucleic acid delivery systems were known to those skilled in the art at the time the invention was made. These delivery systems utilized nucleic acid condensing agents that could be separated into two classes—polycationic lipids and polycationic molecules. Both classes of condensing agents (polycationic carriers) reduce the dimensions of the nucleic acids associated with the polycationic compounds and also protect the nucleic acids from nucleases. Exemplary polycationic carriers discussed in the reference include polycationic lipids, polycationic proteins such as histones or protamines, chemically synthesized compounds such as the poly-amino-acids, polylysine, polyarginine or polyhistidine or polyethylenimine (PEI) (see page 481, column 1, paragraphs 1-2). Ogris *et al.* further indicate that strategies existed for reducing non-specific interactions of the polycationic carrier/nucleic acid complex with blood components and to enable the circulation of complexed DNA in the bloodstream. These strategies included “shielding” the complex surface with hydrophilic polymers, such as (poly)ethylene glycol (PEG), (poly)hydroxypropylmethacrylamide (pHPMA) or (poly)vinylpyrrolidine (see page 481, column 1).

Ogris *et al.* further teach that the non-viral drug delivery systems discussed can be actively targeted to specific cells via a variety of targeting agents (including transferrin, EGF and antibodies specific for cell surface markers (page 481, column 2)). Ogris *et al.* also recognize that synthetic gene-transfer particles still faced several obstacles for delivery of nucleic acids to the cell cytoplasm and/or nucleus. As noted in the reference, “[a]fter internalization by receptor-mediated or adsorptive endocytosis, the vector is enclosed within the endosomal or lysosomal membrane and is, therefore, separated from the cytoplasm ...” (see paragraph bridging pages 481-482). However, Ogris *et al.* also teach that those skilled in the art had tools and strategies for dealing with this obstacle. For

example, melittin and various known viral peptides could be coupled to the particles to facilitate escape from the endosomal vesicles. Alternatively, particles composed of PEI were recognized to have an intrinsic mechanism that enables the release of endocytosed nucleic acids into the cytoplasm under certain conditions. The buffering capacity of the PEI molecules can hamper the acidification of the endosome, causing it to burst (see page 482, column 1).

Ogris *et al.* also indicate that several groups have utilized such polycationic nucleic acid complexes to target distant tumors after systemic application of particulate gene delivery systems (see Table 1). One such system was based on PEI and transferrin, and complexes were shielded with PEG. Another system used a liposomal system known as stabilized plasmid–lipid particle (SPLP), which contained PEG as a shielding agent. This system enabled circulation of the particles in the bloodstream for >6 h after systemic tail-vein injection and tumor-targeted gene expression in subcutaneous growing B16 mouse melanoma. Yet another system utilized a lipid-based particle and used transferring as both the shielding agent and the targeting agent. Additionally, a polyplex system based on a linear PEI derivative shielded with transferring and another polyplex system based on PEI and using EGF as a targeting agent successfully delivered nucleic acids to HUH7 hepatoma established in nude mice. Ogris *et al.* also discuss another delivery system that combined both polycationic carrier molecules (which condense DNA within an inner core) and cationic lipid (which coats the core’s surface with a lipid film) enabled the efficient delivery of therapeutic genes to ovarian carcinoma after tail-vein injection in nude mice (see page 482, column 2 through page 483, column 1 “Predominant gene expression in tumors”).

Finally, Applicants note the Office Action argues that the previously submitted arguments pertaining to Table 1 of Opalinska *et al.* were not found persuasive on the basis that the vast majority of the patients treated by systemic administration of a variety of nucleic acid-based drugs did not show disease remission. Accordingly, the Office Action argues, it was far more likely than not that such drugs could not be used to treat disease via systemic administration. In this regard, Applicants note that the vast majority of the clinical trials were phase I clinical trials devoted to assessing the safety and tolerance of the administered nucleic acid molecules to the subject being treated, yet these initial studies noted, in many cases, some evidence of disease remission or treatment. For the

convenience of the Examiner, a number of the references cited in the table utilizing intravenous administration of antisense nucleic acids are provided with this response in the IDS.

Waters *et al.*, *J. Clin. Oncol.* (2000)18:1812-1823, indicate “[a]lthough this was primarily a safety study, there was evidence for clinical antitumor activity and for specific downregulation of Bcl-2 protein in target tissues. Three patients with low-grade lymphoma had an objective reduction in overall tumor bulk after treatment, and one of them had a complete remission that has been sustained for 36 months. Although spontaneous remissions are known to occur in low-grade lymphoma, it is extremely unlikely that this would happen by chance within a few weeks of antisense therapy, particularly in heavily pretreated patients” (see page 1819, column 1). Additionally, the reference indicates in its “Purpose” and “Conclusions” sections (on page 1812) that its purpose was to “evaluate the pharmacokinetics and toxicity of an antisense oligonucleotide targeting bcl-2 in patients with non-Hodgkin’s lymphoma (NHL) and to determine efficacy using clinical and biologic end points” and its conclusion was that “Bcl-2 antisense therapy is feasible and shows potential for antitumor activity in NHL. Downregulation of Bcl-2 protein suggests a specific antisense mechanism.”

Jansen *et al.* (*Lancet*, 2000, 356:1728-1733) also discuss a phase I-II clinical study that investigated the combination of an antisense nucleic acid in combination with dacabazine for the treatment of advanced malignant melanoma expressing BCL2 (see Summary). Additionally, the discussion at page 1731 indicates that the primary aim of the study was to assess the toxicity of augmerosen combined with full-dose dacarbazine therapy. Jansen *et al.* also report that the nucleic acids were administered as a continuous intravenous infusion for 14 days by an ambulatory infusion pump through a central venous line (see Methods, page 1729, column 1, paragraph 2). In the discussion at page 1732, column 2, Jansen *et al.* state: “Most of our patients had progressive metastatic disease on entry, after failure of dacarbazine-containing regimens or after other standard treatments for metastatic melanoma. Even so, antitumour responses were noted in six of the 14 patients and stabilisation of disease was observed in two others. The estimated median survival of all 14 patients now exceeds 12 months.”

Yacyshyn *et al.* (*Gastroenterology*, 1998, 114:1133-1142) reported on the treatment of Crohn's Disease using intravenously administered antisense oligonucleotides. As indicated in the Background and Aims section, the aim of the study was to:

... obtain preliminary assessment of tolerability, pharmacology, and efficacy of ISIS 2302 in Crohn's disease. *Methods:* Twenty patients with active, steroid treated Crohn's disease were randomized (3:1, ISIS 2302 to placebo) to receive over 26 days 13 intravenous infusions of ISIS 2302 (0.5, 1, or 2 mg/kg) or saline placebo in a double-blinded study. The patients were followed up for 6 months. *Results:* At the end of treatment, 47% (7 of 15) of ISIS 2302-treated and 20% (1 of 5) of the placebo-treated patients were in remission (Crohn's Disease Activity Index [CDAI] < 150). At the end of month 6, 5 of these 7 ISIS 2302-treated remitters were still in remission, and a 6th patient had a CDAI of 156. Corticosteroid usage was significantly lower ( $P = 0.0001$ ) in the ISIS 2302-treated patients. These findings were corroborated by significant increases in  $\beta_7$  and  $\alpha_d$  bearing CD3<sup>+</sup> peripheral blood lymphocytes and by decreases in intestinal mucosal ICAM-1 expression during the treatment period. *Conclusions:* ISIS 2302 seems to be a well-tolerated and promising therapy for steroid treated Crohn's disease.

Yacyshyn *et al.* also indicate that "This study represents the first report of clinical, peripheral blood, and intestinal immune activity for a systemically administered antisense compound. ISIS 2302 appears to be a promising and well-tolerated new therapeutic approach to Crohn's disease, producing a significant steroid-sparing effect while inducing highly durable remissions in 6 of 15 treated patients in this small study" (see page 1141, first full paragraph).

Nemunaitis *et al.*, *J. Clin. Oncol.*, 1999, 17: 3586-3595, conducted a Phase I trial to determine the maximum-tolerated dose (MTD) and pharmacologic behavior of ISIS 3521 (see "Purpose", page 3586) and indicate that "[t]he results of this phase I trial demonstrate that, despite prior concerns surrounding the use of antisense oligonucleotides to treat cancer, such an approach is in fact feasible and promising" (see page 3594, column 2, paragraph 1).

Cunningham *et al.*, *Cancer*, 2001, 92:1265-1271, reported on an initial clinical study of the safety and tolerability of an intravenous infusion of ISIS 2503 in patients with advanced cancer. This article indicated that ISIS 2503, an antisense oligonucleotide against H-ras, was well tolerated as a single agent at doses up to 10.0 mg/kg/day by 14-day continuous intravenous infusion. Several patients had stabilization of disease, suggesting that ISIS 2503 had some tumor growth inhibitory

effects and future trials of ISIS 2503 in combination with chemotherapy should be considered (see page 1265, Background and Conclusions).

Cunningham *et al.*, *Clin. Cancer Res.*, 2000, 6:1626–1631, reported on an initial study of the safety and tolerability of an i.v. infusion of ISIS 5132 in patients with advanced cancer. This reference, too, reported that “ISIS 5132, an antisense oligonucleotide against c-raf, was well tolerated at doses up to and including 4.0 mg/kg/day by 21-day continuous i.v. infusion and demonstrated antitumor activity at the doses tested” (see Abstract).

Finally, Yuen *et al.*, *Clin. Cancer Res.*, 1999, 5:3357–3363, reported on a Phase I study that sought to characterize the safety profile and to determine the maximum tolerated dose of antisense to PKC- $\alpha$  when administered by continuous infusion in patients. Yuen *et al.* reported that “[e]vidence of tumor response lasting up to 11 months was observed in three of four patients with ovarian cancer. The recommended dose of ISIS 3521 for Phase II studies is 2.0 mg/kg/day when given over a period of 21 days. Side effects are modest and consist of thrombocytopenia and fatigue. Evidence of antitumor activity provides the rationale for Phase II studies in ovarian cancer and other malignancies” (see Abstract).

Accordingly, Applicants respectfully submit that the cited clinical trials reported in Opalinska *et al.* actually support a finding that one skilled in the art would find that it was far more likely than not that such drugs could be used to treat disease via systemic administration. Thus, it is respectfully submitted that those skilled in the art would have been able to deliver the claimed siRNA molecules to target cells utilizing known techniques and carrier molecules without undue experimentation and reconsideration and withdrawal of the rejection of record is respectfully requested.

It should be understood that the amendments presented herein have been made solely to expedite prosecution of the subject application to completion and should not be construed as an indication of Applicants’ agreement with or acquiescence in the Examiner’s position. Applicants expressly reserve the right to pursue the invention(s) disclosed in the subject application, including any subject matter canceled or not pursued during prosecution of the subject application, in a related application.

In view of the foregoing remarks and amendments to the claims, Applicants believe that the currently pending claims are in condition for allowance, and such action is respectfully requested.

The Commissioner is hereby authorized to charge any fees under 37 CFR §§1.16 or 1.17 as required by this paper to Deposit Account No. 19-0065.

Applicants invite the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,

/FRANK C. EISENSCHENK/

Frank C. Eisenschenk, Ph.D.  
Patent Attorney  
Registration No. 45,332  
Phone No.: 352-375-8100  
Fax No.: 352-372-5800  
Address: P.O. Box 142950  
Gainesville, FL 32614-2950

FCE/sl

Attachment: Supplemental Information Disclosure Statement